

WHAT IS CLAIMED IS:

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1 1. An isolated infectious chimeric parainfluenza virus (PIV)  
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large  
3 polymerase protein (L), and a partial or complete human parainfluenza virus 3 JS (HPIV3  
4 JS) vector genome or antigenome combined with one or more heterologous gene(s) or  
5 genome segment(s) encoding one or more antigenic determinant(s) of HN and/or F  
6 glycoproteins of HPIV1 and/or HPIV2 to form a chimeric PIV genome or antigenome.

1 2. The chimeric PIV of claim 1, wherein said one or more  
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are  
3 added adjacent to or within a noncoding region of the partial or complete HPIV3 JS  
4 vector genome or antigenome.

1 3. The chimeric PIV of claim 1, wherein said one or more  
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are  
3 substituted for one or more counterpart gene(s) or genome segment(s) in a partial HPIV3  
4 JS vector genome or antigenome.

1 4. The chimeric PIV of claim 1, wherein said one or more antigenic  
2 determinant(s) is/are selected from HPIV1 HN and F glycoproteins and antigenic  
3 domains, fragments and epitopes thereof.

1 5. The chimeric PIV of claim 4, wherein one or more HPIV1 gene(s)  
2 or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic  
3 domain(s), fragment(s) or epitope(s) thereof is/are substituted within the partial or  
4 complete HPIV3 JS vector genome or antigenome.

1 6. The chimeric PIV of claim 5, wherein both HPIV1 genes encoding  
2 HN and F glycoproteins are substituted for counterpart HPIV3 JS HN and F genes in a  
3 partial HPIV3 JS vector genome or antigenome.

1 7. The chimeric PIV of claim 6, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 present within HPIV3 JS *cp45* selected from mutations specifying an amino acid  
4 substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of

5 JS *cp45*; in the N protein at a position corresponding to residues Val96 or Ser389 of JS  
6 *cp45*, in the C protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide  
7 substitution in a 3' leader sequence of the chimeric virus at a position corresponding to  
8 nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene start sequence at a  
9 position corresponding to nucleotide 62 of JS *cp45*

1 8. The chimeric PIV of claim 1, wherein one or more HPIV2 gene(s)  
2 or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic  
3 domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the  
4 partial or complete HPIV3 JS vector genome or antigenome.

1 9. The chimeric PIV of claim 6, wherein a plurality of heterologous  
2 genes or genome segments encoding different antigenic determinants of HPIV1 and/or  
3 HPIV2 are added to or incorporated within the partial or complete HPIV3 JS vector  
4 genome or antigenome.

1 10. The chimeric PIV of claim 9, wherein said plurality of  
2 heterologous genes or genome segments encode antigenic determinants from both HPIV1  
3 and HPIV2 and are added to or substituted within a partial or complete HPIV3 JS vector  
4 genome or antigenome.

1 11. The chimeric PIV of claim 10, wherein one or more HPIV1 gene(s)  
2 or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic  
3 domain(s), fragment(s) or epitope(s) thereof and one or more HPIV2 gene(s) or genome  
4 segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic domain(s),  
5 fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or  
6 complete HPIV3 JS vector genome or antigenome.

1 12. The chimeric PIV of claim 11, wherein both HPIV1 genes  
2 encoding HN and F glycoproteins are substituted for counterpart HPIV3 JS HN and F  
3 genes to form a chimeric JS HPIV3-1 vector genome or antigenome which is further  
4 modified by addition or incorporation of one or more gene(s) or gene segment(s)  
5 encoding one or more antigenic determinant(s) of HPIV2.

1                   13.     The chimeric PIV of claim 12, wherein a transcription unit  
2     comprising an open reading frame (ORF) of an HPIV2 HN gene is added to or  
3     incorporated within the chimeric JS HPIV3-1 vector genome or antigenome.

1                   14.     The chimeric PIV of claim 13 selected from JS rPIV3-1.2HN, or JS  
2     rPIV3-1*cp*45.2HN.

1                   15.     The chimeric PIV of claim 1, wherein the chimeric PIV genome or  
2     antigenome is attenuated by addition or incorporation of one gene or cis-acting regulatory  
3     element from a bovine PIV3 (BPIV3).

1                   16.     The chimeric PIV of claim 1, wherein the chimeric PIV genome or  
2     antigenome incorporates one or more heterologous, non-coding non-sense polynucleotide  
3     sequence(s).

1                   17.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2     antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or  
3     epitopes from both HPIV3 JS and HPIV1 or HPIV2.

1                   18.     The chimeric PIV of claim 17, wherein the heterologous genome  
2     segment encodes a heterologous glycoprotein ectodomain which is substituted for a  
3     corresponding glycoprotein ectodomain in the vector genome or antigenome.

1                   19.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2     antigenome is modified by introduction of an attenuating mutation involving an amino  
3     acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

1                   20.     The chimeric PIV of claim 19, wherein phenylalanine at position  
2     456 of the HPIV3 L protein is substituted by leucine.

1                   21.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2     antigenome incorporates at least one and up to a full complement of attenuating mutations  
3     present within HPIV3 JS *cp*45.

1                   22.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2     antigenome incorporates at least one and up to a full complement of attenuating mutations  
3     specifying an amino acid substitution in the L protein at a position corresponding to

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Sub. B2  
4 Tyr<sub>942</sub>, Leu<sub>992</sub>, or Thr<sub>1558</sub> of in JS *cp45*; in the N protein at a position corresponding to  
5 residues Val<sub>96</sub> or Ser<sub>389</sub> of JS *cp45*, in the C protein at a position corresponding to Ile<sub>96</sub> of  
6 JS *cp45*, in the F protein at a position corresponding to residues Ile<sub>420</sub> or Ala<sub>450</sub> of JS  
7 *cp45*, in the HN protein at a position corresponding to residue Val<sub>384</sub> of JS *cp45*, a  
8 nucleotide substitution in a 3' leader sequence of the chimeric virus at a position  
9 corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene  
10 start sequence at a position corresponding to nucleotide 62 of JS *cp45*.

1 23. The chimeric PIV of claim 21, wherein the chimeric genome or  
2 antigenome includes at least one attenuating mutation stabilized by multiple nucleotide  
3 changes in a codon specifying the mutation.

1 24. The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s)  
3 encoding one or more respiratory syncytial virus (RSV) F and/or G glycoprotein(s) or  
4 immunogenic domain(s), fragment(s), or epitope(s) thereof.

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~~25. The chimeric PIV of claim 1 which is a virus.~~

1 26. The chimeric PIV of claim 1 which is a subviral particle.

1 27. A method for stimulating the immune system of an individual to  
2 induce protection against parainfluenza virus (PIV) which comprises administering to the  
3 individual an immunologically sufficient amount of the chimeric PIV of claim 1  
4 combined with a physiologically acceptable carrier.

1 28. The method of claim 27, wherein the chimeric PIV is administered  
2 in a dose of  $10^3$  to  $10^7$  PFU.

1 29. The method of claim 27, wherein the chimeric PIV is administered  
2 to the upper respiratory tract.

1 30. The method of claim 27, wherein the chimeric PIV is administered  
2 by spray, droplet or aerosol.

1 31. The method of claim 27, wherein the chimeric PIV elicits an  
2 immune response against one or both of HPIV1 and HPIV2.

1                   32.     The method of claim 27, wherein the chimeric PIV elicits a  
2 polyspecific immune response against multiple HPIVs.

1                   33.     The method of claim 27, wherein the chimeric PIV and a second  
2 recombinant PIV are administered sequentially or simultaneously to elicit a polyspecific  
3 immune response.

1                   34.     An immunogenic composition to elicit an immune response against  
2 parainfluenza virus (PIV) comprising an immunogenically sufficient amount of the  
3 chimeric PIV of claim 1 in a physiologically acceptable carrier.

1                   35.     The immunogenic composition of claim 34, formulated in a dose of  
2  $10^3$  to  $10^7$  PFU.

1                   36.     The immunogenic composition of claim 34, formulated for  
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1                   37.     The immunogenic composition of claim 34, wherein the chimeric  
2 PIV elicits an immune response against one or more virus(es) selected from HPIV1,  
3 HPIV2 and HPIV3 JS.

1                   38.     The immunogenic composition of claim 34, wherein the chimeric  
2 PIV elicits an immune response against HPIV3 JS and another virus selected from HPIV1  
3 and HPIV2.

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1                   39.     An isolated polynucleotide comprising a chimeric parainfluenza  
2 virus (PIV) genome or antigenome which includes a partial or complete human  
3 parainfluenza virus 3 JS (HPIV3 JS) vector genome or antigenome combined with one or  
4 more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
5 determinant(s) of HN and/or F glycoproteins of one or both of HPIV1 and HPIV2 to form  
6 a chimeric PIV genome or antigenome.

1                   40.     The isolated polynucleotide of claim 39, wherein said one or more  
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are  
3 added adjacent to or within a noncoding region of the partial or complete HPIV3 JS  
4 vector genome or antigenome.

1                   41.     The isolated polynucleotide of claim 39, wherein said one or more  
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are  
3 substituted for one or more counterpart gene(s) or genome segment(s) in a partial PIV  
4 vector genome or antigenome.

1                   42.     The isolated polynucleotide of claim 39, wherein the chimeric  
2 genome or antigenome is attenuated by incorporation of one gene or cis-acting regulatory  
3 element from a bovine PIV3 (BPIV3).

1                   43.     The isolated polynucleotide of claim 39, wherein the chimeric  
2 genome or antigenome encodes a chimeric glycoprotein having antigenic domains,  
3 fragments, or epitopes from two or more different HPIVs.

1                   44.     The isolated polynucleotide of claim 39, wherein the chimeric  
2 genome or antigenome is further modified by incorporation of an attenuating mutation  
3 involving an amino acid substitution of phenylalanine at position 456 of the HPIV3 L  
4 protein.

1                   45.     The isolated polynucleotide of claim 39, wherein phenylalanine at  
2 position 456 of the HPIV3 L protein is substituted by leucine.

1                   46.     The isolated polynucleotide of claim 39, wherein the chimeric  
2 genome or antigenome incorporates at least one and up to a full complement of  
3 attenuating mutations present within HPIV3 JS *cp45*.

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~~1                   47.     The isolated polynucleotide of claim 39, wherein the chimeric  
2 genome or antigenome incorporates one or more heterologous gene(s) or genome  
3 segment(s) encoding one or more respiratory syncytial virus (RSV) F and G  
4 glycoprotein(s) or immunogenic domain(s), fragment(s), or epitope(s) thereof~~

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1                   48.     A method for producing an infectious attenuated chimeric  
2 parainfluenza virus (PIV) particle from one or more isolated polynucleotide molecules  
3 encoding said PIV, comprising:  
4                   expressing in a cell or cell-free lysate an expression vector comprising an  
5 isolated polynucleotide comprising a partial or complete human parainfluenza virus 3 JS

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6 (HPIV3 JS) vector genome or antigenome combined with one or more heterologous  
7 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of HN  
8 and/or F glycoproteins of HPIV1 and/or HPIV2 to form a chimeric PIV genome or  
9 antigenome, and PIV N, P, and L proteins.

1 49. The method of claim 48, wherein the chimeric PIV genome or  
2 antigenome and the N, P, and L proteins are expressed by two or more different  
3 expression vectors.

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1 50. An expression vector comprising an operably linked transcriptional  
2 promoter, a polynucleotide sequence which includes a partial or complete human  
3 parainfluenza virus 3 JS (HPIV3 JS) vector genome or antigenome combined with one or  
4 more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
5 determinant(s) of HN and/or F glycoproteins of HPIV1 and/or HPIV2 to form a chimeric  
6 PIV genome or antigenome, and a transcriptional terminator.

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